

**Formulation and Characterization of Orally Dissolving Thin Films
Containing the German Cockroach *Blatella germanica*
(Bla g 2) Allergen**

By

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ABSTRACT

Allergy and asthma are the most common diseases associated with cockroach infestation of houses in the United States and other parts of the world. Allergy to cockroach has been recognized as a significant health problem world-wide, and is often coupled with urban development and inner-city environments. In the United States the prevalence of cockroach allergy ranges from 17% to 41% in both adults and children, and allergic diseases constitute the most common chronic diseases of childhood and represent approximately 25% of children's sick visits to pediatricians.

The principal domestic cockroach species are *Blattella germanica* (German cockroach) and *Periplaneta americana* (American cockroach). The German cockroach is the most common species associated with allergies in the US and Europe, and the American cockroach is found more commonly in South America and some Asian countries. Both species produce several potent allergens, including Bla g 2, an inactive aspartic proteinase; Bla g 4, a calycin, Bla g 5, a glutathione-S-transferase, and the group 1 cross-reactive allergens Bla g 1 and Per a 1. Of these, Bla g 2 appears to be an especially potent allergen, which elicits IgE responses at exposure levels that are hundreds fold lower than other cockroach allergens.

Currently subcutaneous injection immunotherapy is the only potentially curative treatment available for allergy, but has clear disadvantages and is not acceptable to many children. Also treatment by injection can carry great risks such as anaphylactic shock, especially for food allergies. Our research group is actively involved in the development of sublingual immunotherapy (SLIT). In this work, a formulation of orally dissolving thin film has been developed and characterized. The film allows for higher and more effective dosing, and its

mucoadhesiveness prolongs the effect of the allergen and leads to enhanced immunotherapy for asthma.

It is found in this work that the formulation developed for the treatment of German cockroach allergy incorporates a significant amount of the Bla g 2 allergen. Its potency and stability have been characterized by enzyme-linked immunosorbent assay (ELISA), and other pharmacological properties are evaluated by spectroscopic methods. This SLIT methodology has the potential to change the face of immunotherapy for both food and inhalant allergies in adults and children. This essay will provide an overview of the development and characterization of Bla g 2 SLIT films.

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Introduction:

Inner city residents suffer disproportionate asthma incidences compared to their suburban counterparts in the United States. Although many factors may be responsible for these disparities, there is a growing body of evidence to show that indoor environments play a key role in asthma control and other asthma related health issues in inner-city populations. The term "inner-city" refers to impoverished urban neighborhoods with dilapidated and poorly kept housing, where there is an increased chance for pest infestations and "asthmagenic" exposures. Among the major categories of indoor allergens are endotoxins, pollutants, and biologics including those from dust mites, pets, rodents and cockroaches². Studies have found that more than half of asthma cases in the United States is caused by allergic sensitization³, and in one inner-city study of children with poorly controlled asthma, a staggering 94% of children showed evidence of allergic sensitization to some form of indoor allergen². While majority of the previously listed indoor allergens can be found in inner-city homes across the country, there is a geographical pattern to the distribution of allergens^{4,5,6}. For example, Dust mite is the predominant indoor allergen in southern US while cockroach is more often found in northeastern regions of the country. Therefore it is important to understand the geographical relevance of the allergens and provide tailored testing as well as treatment to patients.

In this current study we chose the German cockroach as the target allergen of our sublingual immunotherapy (SLIT) development. Many species of cockroaches generate a wide group of allergens with diverse structural variations and biological functions. They are released into the environment through the digestive tract of the insect or excretion with feces. The allergenic particulates are large and settles onto surfaces easily. Usually disturbance of the dust is

required for sensitization, however in the case of the cockroach allergen, miniscule amounts of unsettled dusts is sufficient to induce allergic sensitization⁹.

Multiple allergens from various species of cockroaches have been characterized, and eight groups of cockroach allergens have been added to the World Health Organization (WHO) list of Allergen Nomenclature, including the German cockroach *B. germanica* allergens Bla g 1, Bla g 2, Bla g 4, Bla g 5, Bla g 6, Bla g 7 and Bla g 8, and the American cockroach *P. americana* allergens Per a 1, Per a 2, Per a 3, Per a 6 and Per a 7⁴. **(Table 1)** Contrary to its name, the American cockroach is predominantly found in South America and some Asian countries, whereas the German cockroach is mainly found in the United States⁹. For this reason current inner-city asthma studies have primarily focused on the German cockroach (Bla g) allergens, and more specifically, Bla g 2. Bla g 2 is a very potent allergen that elicits more than 60% of IgE antibody response at exposure levels 10-100 fold lower than that of other common indoor allergens³. The Bla g 2 allergen was successfully cloned in 1995 and in 2005 Gustchina et al¹⁰ solved its crystal structure.

Bla g 2 is a 36 kDa glycoprotein that shares homology with the aspartic protease family¹⁰ **(Figure 1)**. Aspartic proteases are enzymes that use aspartate to catalyze target substrates. These enzymes share two conserved aspartate residues in the active site which is commonly inhibited by pepstatin. The crystal structure of Bla g 2 shows a bilobal shape that is typical of aspartic proteases, and a cleft between them that contains the catalytic site. Sequence similarity between Bla g 2 and other aspartic proteases is close. For example, there is a 52% similarity between Bla g 2 to pepsin, chymosin and renin, and 49% between it and proteinase A.¹⁰ **(Figure 2)** However, Bla g 2 differs from this family of protein in several ways. First, Bla g 2 has a metal binding site that is occupied by a tightly bound zinc cation.¹⁰ **(Figure 3)** Three out of the four residues that

form this binding site are unique to the sequence of Bla g 2 and have not been found in other related cockroach allergens.¹⁰ Second, five disulfide bridges are found in the structure of Bla g 2, and only two of them are conserved within the pepsin family while the remaining three do not have structural analogs in other identified aspartic proteases. Similarly, a disulfide bridge found in other aspartic proteases is absent in the Bla g 2 structure. Substitutions of the amino acid glycine by serine or threonine, as well as insertions in the active site renders this Bla g 2 to an inactive aspartic protease.¹⁰

The stability of the Bla g 2 allergen can be attributed to an increased number of disulfide bridges and the incorporation of the zinc cation. In allergic sensitization, the stability of the tertiary structure of the allergen is crucial to IgE recognition. Disruption to the native structure leads to reduced sensitivity. Bla g 2 has five disulfide bridges instead of the two or three usually found in other aspartic proteases. The number of disulfide bridges in a protein molecule often correlates with its stability, and in addition the zinc cation strengthens the two neighboring secondary structures of the protein. The increased structural stability can influence the persistence of the allergen in the environment, and chronic exposure to such a stable allergen can explain why even at low doses ($< 1\mu\text{g/g dust}$)⁹ it is still able to elicit an allergic reaction.

The National Cooperative Inner City Asthma Studies declares that 37% of children with asthma in the United States have been exposed and show sensitization to cockroach allergens⁹. The highest values of cockroach exposure of up to 81% are found in Bronx, New York and Dallas, Texas⁹. At least 50% of inner-city homes have been shown to have clinically relevant levels of cockroach allergen^{4,7}, and 30% of suburban homes show detectable levels of the allergen^{5,8}. Numerous studies over decades have demonstrated that the combination of cockroach allergy and cockroach exposure is one of the most important factors leading to high morbidity in

inner-city children with asthma¹¹. The National Institute of Allergy and Infectious Diseases sponsors the Inner-City Asthma Consortium (ICAC), whose main goal is to develop immune-based treatments for cockroach allergy that focuses on the root of the disease instead of its management. There are significant risks associated with allergy treatments especially in small children, one of which is the potential for anaphylactic shock during immunotherapy¹¹. The most common immunotherapy in the United States is subcutaneous injectable immunotherapy (SCIT)¹². The major drawbacks to this therapy are acceptability and safety. SCIT requires regular injections over a prolonged period of time, and this leads to only a small fraction of children who could potentially benefit from this therapy to be treated with it. Also, systemic allergic reactions are relatively common with SCIT treatment, especially for respiratory allergies such as cockroach, occurring in one out of nine test subjects.¹³

Recently the use of liquid extracts were tested. These extracts are the most common form of sublingual immunotherapy in the US in studies of both food and respiratory allergies, but they cannot be easily concentrated to the doses likely to be effective in immunotherapy. A previous dose at 3.3µg was evaluated but after 6 months of treatment there was no change in allergen specific IgG 4 [unpublished data], an antibody that is postulated to be important for the mechanism of immunotherapy.

A dissolving film could be a viable alternative to both SCIT treatment and also treatment by liquid allergen extracts. Film drug delivery is a method of delivering drugs using a thin film that dissolves when in contact with liquid (eg. saliva), and delivers the active compound to the systemic circulation. A thin film formulation allows for safer and more patient-compliant therapy, more effectively stabilizes the enzymatic activity of the allergenic protein, and also delivers a much higher dose.

Commonly referred to as sublingual immunotherapy (SLIT), thin film formulations offer the potential to improve the onset of action, lower the required dosage, and enhance the safety and efficacy of the treatment. Most oral dosage forms enter the blood stream through the gastrointestinal (GI) tract, which subjects the drug first to degradation in the acidic environment of the stomach, then bile, digestive enzymes, and all other first pass effects. A higher initial dosage is therefore required to achieve the desired end result, and due to the traveling time in the GI tract, onset of action can be significantly delayed. Sublingual dosing avoids these issues to yield quicker onsets and lower doses. Also, the film's ability to dissolve without water, and elimination of the needle naturally makes it an attractive drug delivery regimen for pediatric patients where compliance can be difficult.¹³

Cockroach allergy is a key contributor to asthma morbidity in inner-city children. Pilot studies lead by the Johns Hopkins School of Medicine document the immune responses of inner-city residents to cockroach allergen and provide directions for the development of immunotherapy which ultimately lead to the research that forms the topic of this essay. We have developed a SLIT film for the treatment against Bla g 2 allergies. The films are formulated with 25ug of Bla g 2 allergen per single dose, nearly 10 fold higher compared to using only liquid extracts, and without the safety drawbacks of SCIT treatments. We characterized the film formulation based on its appearance and physical properties, confirmed allergen content using enzyme-linked immunosorbent assay (ELISA), and tested *in vitro* dissolution and disintegration via spectroscopic methods. Our results show that the thin film formulation is uniform in allergen content, with allergen release profiles that meet the preset requirements, and also possess superior stability even without chemical modification or treatment.

Orally Dissolving Strips:

The oral route is a convenient method of drug administration because of its ease of dosing, cost effectiveness as well as high levels of patient compliance. It can become problematic however for pediatric and geriatric patients who may have fear of choking or difficulty swallowing. Recently fast dissolving drug delivery systems have gained popularity for their increased convenience, rapid dissolution and disintegration, and self-administration even without water. Various mucoadhesive dosage forms have been investigated, including tablets, gels, ointments, patches and as is seen in this study, quick-dissolving films. An ideal fast dissolving delivery system should possess high formulation stability, transportability, ease of handling and administration, requires no special packaging or storage conditions, have a pleasant taste, and more importantly have a tunable dissolution time. Materials chosen for the formulation of thin films include film-forming water-soluble polymers, plasticizers, the active ingredient, sweetening/flavoring agents, coloring agents, stabilizing or thickening agents. The excipients used should be approved for use in oral pharmaceutical dosage forms by the US Food and Drug Administration (FDA).¹⁴

The first kind of oral strip was developed by Pfizer and marketed under the name Listerine® pocket packs™. Chloraseptic® was the first therapeutic oral thin film which contained 7-benzocaine for the treatment of sore throat. The films are usually formulated to be 1–20cm² in size and drug loading of up to 30 mg.¹⁴ The polymer of choice, along with plasticizers, stabilizing or thickening agents heavily influence the mechanical properties of the film as well as its long term shelf life. A variety of polymers and related excipients are available

for the formulation of orally dissolving strips, also available is a wide array of manufacturing techniques.

A variety of polymers are available for the formulation of fast dissolving oral films. The properties of the selected polymers are crucial to the successful development of the formulation. The principle criteria for the polymer excipients include:¹⁴

- they should be nontoxic and nonirritant with none or minimal impurities;
- they do not affect drug release profile;
- they are devoid of offensive odor or taste;
- they exhibit good wetting and mucoadhesive property;
- they have sufficient peel, shear and tensile strength;
- they are cost effective; and
- they have desirable shelf life.

Table 2 provides a list of the currently available natural and synthetic polymers used in formulating orally dissolving strips. **Tables 3** and **Table 4** represent the quality parameters of these polymers, respectively. Conventional approaches to formulating fast-dissolving films include the following¹⁴, and a brief description for each is given below.

- Solvent casting
- Hot melt extrusion
- Semisolid casting
- Solid dispersion extrusion
- Rolling

Solvent casting

In this method, water-soluble polymers are dissolved in aqueous media at elevated temperatures (60-90°C) under agitation. The polymer solution is mixed with the appropriate concentrations of other excipients such as coloring and flavoring agents and finally the active ingredient is incorporated. Air bubbles can be removed by vacuum or brief sonication. The resulting solution is poured into the mold and allowed to dry. Afterwards, the dried sheets are cut into the desired size.¹⁴

Hot melt extrusion

In the hot melt extrusion method, the initial formulation is formed with the help of carriers to obtain a solid mass and dried. The dried intermediate product is introduced into the extruder and re-melted at the desired temperature. The extrudate is pressed into a film. The main advantage with this method is better content uniformity, since in such a melt sedimentation of one or more of the components is minimal.¹⁴

Semi-solid casting

Semi-solid casting is the preferred method when one of the excipients is an acid insoluble polymer, for example, cellulose acetate phthalate or cellulose acetate butyrate. To begin, the water-soluble polymers are dissolved in aqueous media and the solution is added to the acid

insoluble polymer solution that is prepared separately. The ratio of the acid insoluble polymer to the film-forming polymer should be no greater than 1:4. After mixing, a plasticizer is added to form a gel and to enhance the final film's physical properties. The gel is casted into the film and processed by standard methods.¹⁴

Solid dispersion extrusion

This method involves the solid dispersion of the drug in a melted polymer solution. The drug is first dissolved in a liquid solvent and then added to the melt of the selected polymer. They are mixed thoroughly without removing the liquid solvent to obtain the solid dispersion. The final dispersions are shaped into films.¹⁴

Rolling

In this method, both the drug solution and film-forming polymer are mixed together and the resulting solution or suspension is subjected to a processing roller. The rolled out sheets are dried and cut into the desired shapes and sized.¹⁴

Clearly fast dissolving thin films can be an advantageous approach to oral drug delivery. It promises patient compliance especially in children and for immunotherapy purposes, it is also considered a much safer route of administration compared to the more widely used subcutaneous injection method. The films are easy to formulate, process and scale up. A successful sublingual thin film formulation could potentially change the future of immunotherapy.

Materials and Methods:

Chemicals

Defatted ground cockroach source powder was purchased from Greer, USA. Methocel E 15 LV Premium was obtained from The DOW Chemical Company, Midland, Michigan. Glycerin NF grade was obtained from Spectrum Chemical MFG Corp., and distilled and deionized water used in the formulation and all experimental analyses was obtained through a Milli Q water purification system (Millipore Corp.) All of the above materials were of analytical grade.

The Blat g 2 ELISA kit was purchased from Indoor Biotechnologies. The protocol was modified to be used with anti-rabbit HRP (horseraddish peroxidase), which was obtained from Thermo Scientific. 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS was purchased in its powder form from Invitrogen.

Preparation of Blatellagermanica crude extract

Powdered German cockroach source material was kept frozen at -20°C until used. The allergen solution was prepared by suspending the powdered source material at 20% w/v in deionized water and incubating the suspension at 4°C overnight. Afterwards the suspension was taken out and centrifuged at 3000X g at 4°C for 15 minutes, and collecting the supernatant. The Blat g 2 content in the supernatant was quantitated using ELISA assay.

Preparation of SLIT thin films

A solvent casting method was used to prepare the thin filmstrips used to deliver the cockroach allergen extract. The optimized composition of the film is tabulated in **Table 5**. Cockroach protein extract, Methocel E 15, glycerin, water, and chocolate flavoring agent were mixed in a suitable vial followed by brief, pulsed ultrasonic defoaming to remove the air bubbles in the solution. The solution was cast onto a flat Teflon cast that has dimensions of 2.5cm × 5cm, which was custom-made. The films were dried overnight in a 40°C oven to evaporate the excess water, and then gently peeled off the cast. The edges of the films were trimmed off, and they were cut into 1cm × 2cm strips. Bl a g 2 content in each strip was assayed using ELISA.

Weight and Thickness

The weight and thickness of the 1cm × 2 cm film-strips were measured. Weight was measured by an analytical balance (Mettler Toledo, Inc), while thickness was measured using a calibrated digital Vernier Calipers (Cen-Tech®). The thickness of each strip was evaluated at five different locations (four corners and one center) as shown in the schematic diagram below.¹⁴ The data helps to ascertain the physical uniformity of the films as it is directly related to the accuracy of the dose distribution in the film.



Foldability

Foldability was assessed by folding each 1cm × 2cm film-strip repeatedly at the same place as indicated by the dashed line until breakage occurs or visible cracks began to appear. A total of three strips were tested. The number of times the strip could be folded without breaking or cracking was recorded as its foldability value.



Tensile strength

Tensile strength was measured using a Dynamic Mechanical Analysis instrument (DMA Q800). The instrument was equipped with one fixed clamp and one movable clamp, which extends to induce strain on the specimen. Each film strip was loaded securely into the clamps and pulled at a constant rate of 100 μm per minute to a maximum extension of 2000 μm (10% elongation). The result was recorded on an engineering stress vs strain curve. The highest point of the curve was reported as the ultimate tensile strength, and the slope of the linear portion of the stress vs. strain curve was calculated as the Young's modulus of the specimen.

Water content

To assess the water content of the thin film, they were weighed at the end of the casting process, and then put into separate borosilicate scintillation vials and lyophilized for periods of 12 hours. At the end of each period, the films were extracted and allowed to return to ambient conditions in a desiccator before re-weighing. Afterwards they were placed back into lyophilization. This process was continued until two consecutive weighings did not differ by more than 0.50 mg (1% of total strip weight) per each strip. The difference in initial and end weight was recorded as the absolute water content of the strip.

Surface pH test

The surfaces of the films were wetted slightly with deionized water and its pH measured by bringing the electrode into contact with the wetted surface.

In vitro disintegration test

Disintegration here is referred to as the physical process by which the film dissolves into a solution. Disintegration time was measured for three strips according to a method previously described by Balaet al.¹⁴ Briefly, the film strip was dipped in 25 mL of water in a suitable container. The water was gently agitated by a 1-inch stir bar at 75 rpm. Disintegration time was recorded when the film starts to break apart or disintegrates.

In vitro dissolution test

In vitro dissolution time was measured in accordance to the conditions set out in the United State Pharmacopeia. 900 mL of fresh deionized water was added to a suitable container as the dissolution media. The media temperature was maintained at $37 \pm 0.5^{\circ}\text{C}$. The medium was agitated by a 1-inch Teflon®-coated stir bar rotating at 75 rpm. At time points 10, 15, 20, 25, 30, 45 and 60 minutes, 1 mL aliquots of samples were extracted and measured on an UV/Vis spectrophotometer (Thermo Scientific, NanoDrop, 2000c) at wavelength 300 nm. The resulting absorbance values were correlated with those of a serially-diluted cockroach protein standard.

Content Uniformity

The dosage uniformity of the Bla g 2 allergen was quantitated using the ELISA assay for ten filmstrips. Each film was fully dissolved in 1 mL of water and then pre-diluted 100 fold before they were loaded onto a 96-well microtiter plate for ELISA. The amount of Bla g 2 was quantitated based on the logarithmic fit of the ELISA standard curve. The contents of each of the strip was to be between 85% and 115% and the relative standard deviation is less than or equal to 6.0%. The acceptance value (AV) of the sample preparations much be less than 15%, according to the United States Pharmacopeia. The AV is calculated according to the following equation:

$$AV = | M - X | + ks$$

Equation (1)

where, M is the label claim (100%), X the average (%) of the individual samples, k the acceptability (2.4 for $n = 10$), and s is the standard deviation (SD) of the sample set.

Results:

Preparation of Blatella germanica crude extract

The Bla g 2 concentration in the aqueous extract solution was measured for three separate protein extracts and the average amount of Bla g 2 extracted from the crude source material was determined to be $155.8 \mu\text{g/mL} \pm 23.7 \mu\text{g/mL}$. Percent relative standard deviation (% RSD) is defined as the absolute value of the coefficient of variation. It is widely used to express the precision and repeatability of an assay. The % RSD of this assay was 15.2%. Based on our past experiences and the inherent variability in ELISA for allergens, a % RSD of less than equal to 20% is considered an acceptable level of variation in the assay data.

Preparation of SLIT thin films

The optimized composition of the thin film incorporated 25 μg per strip of the potent German cockroach Bla g 2 allergen in each of the 1 cm \times 2 cm strip, as determined by ELISA assay. The selected composition demonstrated the most satisfactory physical properties. The films dried tack-free, easily peeled from the cast, could be cleanly cut into desired dimensions, and maintained superior flexibility even post-drying (**Figure 4**).

Weight and Thickness

The average weight of the filmstrips were found to be $41.7 \text{ mg} \pm 3.4 \text{ mg}$ (Mean \pm SD, $n=10$), with % Relative Standard Deviation (RSD) of 8.2%. Typically variance of $<10\%$ was considered an adequate level of reproducibility. The weight of blank filmstrips averaged $24.8 \text{ mg} \pm 2.2 \text{ mg}$, with % RSD of 8.9%. As expected, strip weight increased when the more dense allergen extract was incorporated.

Thickness measurements were taken for ten strips at the five designated locations shown previously. The mean thickness was first calculated for each strip then averaged across all ten samples. The total mean thickness of the strips was determined to be $78.0 \text{ } \mu\text{m} \pm 21.8 \mu\text{m}$ (Mean \pm SD, $n=10$). The filmstrips tended to be thicker near the edges due to the effect of drying. The films were casted using molds fabricated locally with limited selection of size, which constrained our ability to excise the desired dimensions while avoiding edges. We fully anticipate that when this study proceeds to cGMP manufacturing, where much larger molds are employed, these variations in thickness would be significantly reduced. We report here also the thickness at the center of the strips as $93.0 \text{ } \mu\text{m} \pm 13.4 \text{ } \mu\text{m}$, which strengthens our hypothesis that more uniform thickness distribution will be achieved once the edge effect is eliminated.

The thickness of blank filmstrips were also measured, and averaged to be $64.3 \text{ } \mu\text{m} \pm 9.8 \text{ } \mu\text{m}$. This result correlates with the strip weight data. As expected, the blank filmstrips are lighter and thinner compared to the filmstrips incorporating the allergen extract.

Foldability

We also determined the foldability of the filmstrips, to confirm that they possess the necessary flexibility for each maneuver. Multiple strips with the highest protein extract content were tested, as flexibility decreases with increased extract percentage. The average number of folds the films can endure before cracks were observed or complete failure was 11.0 ± 1.0 (Mean \pm SD, $n = 3$).

The foldability of three blank filmstrips were also tested. The blank filmstrips were much more flexible compared to their active counterparts, and the foldability for each of the three tested strips exceeded 100 folds.

Tensile strength

Tensile strength, or more properly, ultimate tensile strength (UTS), is an important mechanical property for thin films. It measures the maximum stress that the film can withstand while being stretched or pulled before failing or breaking. The UTS of the strips with the highest protein loading level (40%) was tested to be $15.0 \text{ MPa} \pm 5.3 \text{ MPa}$. Based on the behavior of the filmstrips we concluded that they possess sufficient physical integrity to allow easy processing and handling during manufacturing, transportation, and storage. A typical stress-strain curve for a filmstrip is shown in **Figure 5**.

The ultimate tensile strength of blank filmstrips were reported as $14.6 \text{ MPa} \pm 1.2 \text{ MPa}$.

Water content

The filmstrips were determined to contain on average $2.9\% \pm 0.8\%$ of water ($n = 8$). Water content is an important criteria as it can directly affect the shelf life of the filmstrips. At

the time this thesis was being prepared, a cGMP-compliant study is in progress to correlate the water content in films and their maximum shelf life.

Surface pH test

Surface pH was recorded for three filmstrips. The average pH was 6.4 ± 0.3 (mean \pm SD), RSD was 4.0%. Surface pH provides guidance on the acidity of the filmstrips after processing. The pH should be neutral or close to neutral as to not cause irritation to the oral mucosa during administration. The obtained pH value conforms to this criterion, as it is comparable to the pH of common drinking water which is between 6.5 to 8.5, according to guidelines by the World Health Organization.

In vitro disintegration test

Disintegration was carried out for three filmstrips using deionized water as the disintegration medium. The time it took for the film to visually break apart were 82, 101 and 115 seconds. The mean disintegration time was 99.3 ± 16.6 seconds or 1.7 ± 0.3 minutes. A disintegration time of lower than 5 minutes was considered satisfactory for this formulation.

In vitro dissolution test

A serially diluted cockroach protein standard is shown in **Figure 6**. Dissolution time was measured for three filmstrips over a period of 45 minutes and the absorbance values were

correlated with the constructed standard curve. The release profiles of the strips are shown in **Figure 7**. Total protein release was achieved within 20 minutes. The expectation for an immediate release formulation is 80% released within 45 minutes, and in this formulation, 80% release was achieved at 15 minutes \pm 3 minutes, which conforms to our expectations for the formulation.

Content Uniformity

Ten strips were randomly selected and their Bla g 2 contents were assayed by ELISA. The acceptance value (AV) was calculated using the method set out in the current United States Pharmacopeia and equation (1).

$$AV = | M - X | + ks$$

The target Bla g 2 content per dosage unit was 25 μ g/filmstrip, which was set as the label claim. RSD was 5.3%, which conforms to the acceptance criteria of less than or equal to 6.0%. The maximum allowed acceptance value for each individual preparation was no more than \pm 15.0%. Based on these values, the tested strips were determined as having acceptable uniformity in content of the Bla g 2 allergen.

Discussion:

Recent studies have highlighted cockroach allergy as an important cause of inner-city asthma. In a survey of over 400 children with asthma in the United States, Rosenstrich et al¹⁹ found that having high levels of the cockroach allergen Bla g 2 in the bedroom is correlated with asthma morbidity if the children living there also had sensitivity to the allergen. But at the same time, the presence of cockroach allergen in the indoor environment is a major risk factor for developing sensitization. Bla g 2 is an especially potent allergen due to its increased number of disulfide bridges and the zinc cation, both features serve to strengthen the tertiary structure of the allergen and preserve its stability in the environment. Therefore sensitization to Bla g 2 can occur even with relatively low amount of exposure²⁰. Cockroaches are ubiquitous, this makes prevention by reducing exposure difficult or even impractical to achieve. In one study, the reduction of mites and cockroach allergens on the bedroom floor was found to correlate with fewer symptom days and less hospital visits due to asthma²¹. Such abatement interventions can become cost-effective in the long run, but the substantial time and resources commitment that is required may not always sustain this maintenance duration. Because of this, allergen immunotherapy has become the major treatment scheme in that it offers the best hope for a long-term reduction in hypersensitivity and improved quality of life.

Allergen immunotherapy is most often carried out using allergen extracts, at least 30 million doses are given each year in the US alone²². These are usually given as subcutaneous injections, which have serious risks associated with them especially for people with extreme sensitivities. Cockroach allergen extracts, along with many others, are nonstandardized allergen extracts, but they constitute the majority of products available in the US²³. They are labeled

either with the weight to volume dilutions of a stock extract, or with protein nitrogen units (PNU), both measurements have little relationship with the potency of the allergen, therefore the exact dosage used in each immunotherapy is difficult to assess²⁵.

The safest approach to immunotherapy is to use extracts and formulations for which the allergen potency has been ascertained. Sublingual immunotherapy (SLIT) has rapidly gained popularity, since it is a noninvasive and efficacious treatment of common food and respiratory allergies²⁴. In this study, our SLIT thin film formulation were formulated as immediate release dosage forms. They contain known amounts of Bla g 2 allergen; and its potency and stability have both been evaluated to ensure they maintain the critical allergen characteristics over time. These films provide an alternative to the current subcutaneous injection in improving patient compliance, ease of self-administration, and better bioavailability.

The commercial allergen source material that we obtained from Greer contains suitably high concentrations of allergen. We optimized the solubilization protocol to ensure consistent levels of protein extraction and therefore high Bla g 2 doses in the films. Methocel® E 15 was chosen to confer mucoadhesive properties to the films. Visual examination showed that the fabricated filmstrips were smooth, lightly transparent with good flexibility, across the entire dose range. Physicochemical characterizations performed on the filmstrips show that they are uniform in weight and thickness, bear stress well, and can maintain physical integrity during bending and pulling.

Dosage uniformity has been an ongoing challenge with nonstandardized allergens such as the cockroach allergens. With most commercial products having label claims in irrelevant units, it carries great risk in immunotherapy when the potency of the formulation cannot be accurately determined. We have established a standardized protocol for the extraction of cockroach soluble

proteins, as well as for the film casting process. The sublingual films we have developed here have been carefully characterized here for their uniformity so that we have full confidence that we can achieve the label claim of 25 µg/strip when the research proceeds to manufacturing and subsequent clinical trials.

Preliminary stability studies conducted for a period of 1 week for the soluble protein extract and 9 weeks for the filmstrip prototypes showed no appreciable degradation in allergen content. This finding is significant because if the Bla g 2 protein is stable in solution, manufacturing of these strips can be done in a much less constrained timeline with no adverse effects to the end product. cGMP compliant stability studies are in progress to assess the stability of the filmstrips for up to one year, which is the anticipated length of the clinical trial.

Cockroach allergy is important in the pathogenesis of asthma in the United States. In this study, we have successfully developed a SLIT thin film formulation using fast dissolving oral thin films to treat patients with hypersensitivity to cockroach. The films exhibit good mechanical strength, consistent potency and adequate stability. While only the cockroach allergen was studied in this report, the SLIT methodology has enormous potential to change the face of immunotherapy for both food and inhalant allergens in the future.

Table 1. Systematic Nomenclature and Functional Properties of Cockroach Allergens⁴

<i>Blattella germanica</i>				
Allergen	M.W.*	Function/homology	Accession number	Reference
Bla g 1	~25-90	Midgut microvilli protein homolog		
Bla g 1.0101	46, 21		AF072219, AF072221	[58]
Bla g 1.0102	25-37		L47595	[57]
Bla g 1.02	56		AF072220	[58]
Bla g 2**	36	Unusual aspartic protease	U28863	[8; 42; 70]
Bla g 4	21	Lipocalin	U40767	[43]
Bla g 5	23	Glutathione S-Transferase	U92412	[44]
Bla g 6	17	Troponin C		
Bla g 6.0101			DQ279092	[47]
Bla g 6.0201			DQ279093	[47]
Bla g 6.0301			DQ279094	[47]
Bla g 7	33	Tropomyosin	AF260897	[49]
Bla g 8		Myosin light chain	DQ389157	[47]
<i>Periplaneta americana</i>				
Allergen	M.W.*	Function/homology	Accession number	Reference
Per a 1	25-45	Midgut microvilli protein homolog		
Per a 1.0101	26		AF072222	[59]
Per a 1.0102	26		U78970	[60]
Per a 1.0103	45		U69957	[93]
Per a 1.0104	31		U69261	[60]
Per a 1.02	51		U69260	[60]
Per a 2	36	Unusual aspartic protease	AY792947	FT Chew [42]
Per a 3	46-79	Arylphorin/hemocyanin		
Per a 3.01	79		L40818	[45]
Per a 3.0201	75		L40820	[45]
Per a 3.0202	56		L40819	[45]
Per a 3.0203	46		L40821	[45]
Per a 6	17	Troponin C	AY792950	FT Chew [47]
Per a 7	33	Tropomyosin		
Per a 7.0101			Y14854	[89]
Per a 7.0102			AF106961	[24]
Per a 9		Arginine kinase		
Per a 9.0101	40		AY563004	[53]

* Molecular weight calculated from sequence.

** The accession number for Bla g 2 in the Protein Data bank is 1YG9.

Table 2. Polymers used in the formulation of fast dissolving films¹⁴

<i>Polymer</i>	<i>Examples</i>
Natural polymer	Pullulan, starch, gelatin, pectin, sodium alginate, maltodextrins, polymerized rosin
Synthetic polymer	Hydroxypropyl methylcellulose, sodium caboxymethylcellulose, polyethylene oxide, hydroxypropyl cellulose, polyvinylpyrrolidone, polyvinyl alcohol, ethyl cellulose

Table 3. Various types of synthetic polymers and their properties¹⁴

<i>Polymer</i>	<i>Water solubility</i>	<i>pH</i>	<i>Moisture (% loss on drying)</i>	<i>Molecular weight (kDa)</i>
Hydroxylpropyl cellulose	Soluble in water	5-8	1.6	50,000-1,250,000
Hydroxylpropyl methylcellulose	Soluble in cold water	5-8	1.6	50,000-1,250,000
Sodium carboxymethylcellulose	Viscous colloidal solution	6-8	10	90,000-700,000
Polyvinyl alcohol	Readily soluble	5-8	5	20,000-200,000
Polyethylene oxide	Readily soluble	8-10	<1	Variable
Kollicoat	> 50% in water	6-7	-	About 45,000

Table 4. Various types of natural polymers and their properties¹⁴

<i>Polymer</i>	<i>Water solubility</i>	<i>pH</i>	<i>Moisture (% loss on drying)</i>	<i>Molecular weight (kDa)</i>
Pullulan	Readily soluble	5-7	6	100-250
Sodium alginate	Slowly soluble, forming viscous solution	7.2	15	-
Pectin	Soluble in water	6-7.2	10	30,000-100,000
Gelatin	Swell in water and soften	3.8-6.0	10	15,000-250,000
Maltodextrine	Swell in water and soften	4-7	6	Variable

Table 5. Optimized compositions of the sublingual immunotherapy thin film developed in this study

<i>Components</i>	<i>Formulation (%w/v)</i>
Defatted cockroach extract	0 – 40
Methocel E15 LV Preimum*	50
Deionized water	49.8 – 9.8
Glycerin	0.15
Chocolate flavoring	0.5

*10% w/v solution

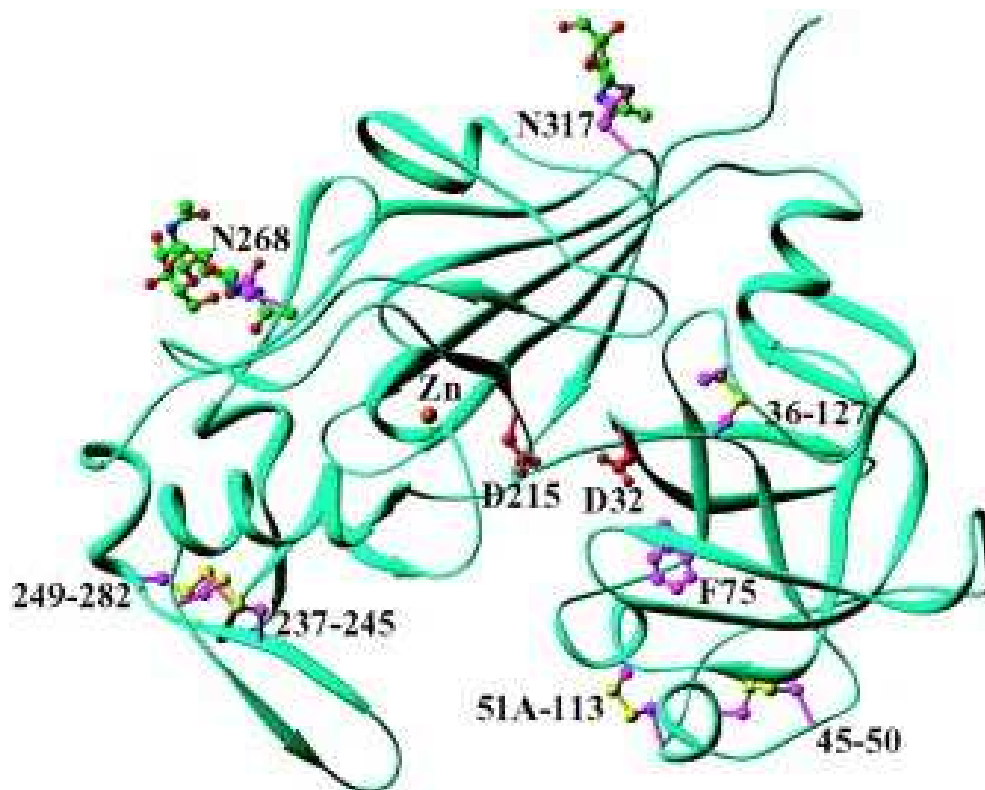


Figure 1. Ribbon representation of the overall fold of Bla g 2. The disulfide bridges and polysaccharides are shown in ball-and-stick representation and their location is marked. The position of Zn is marked as an orange ball.¹⁰

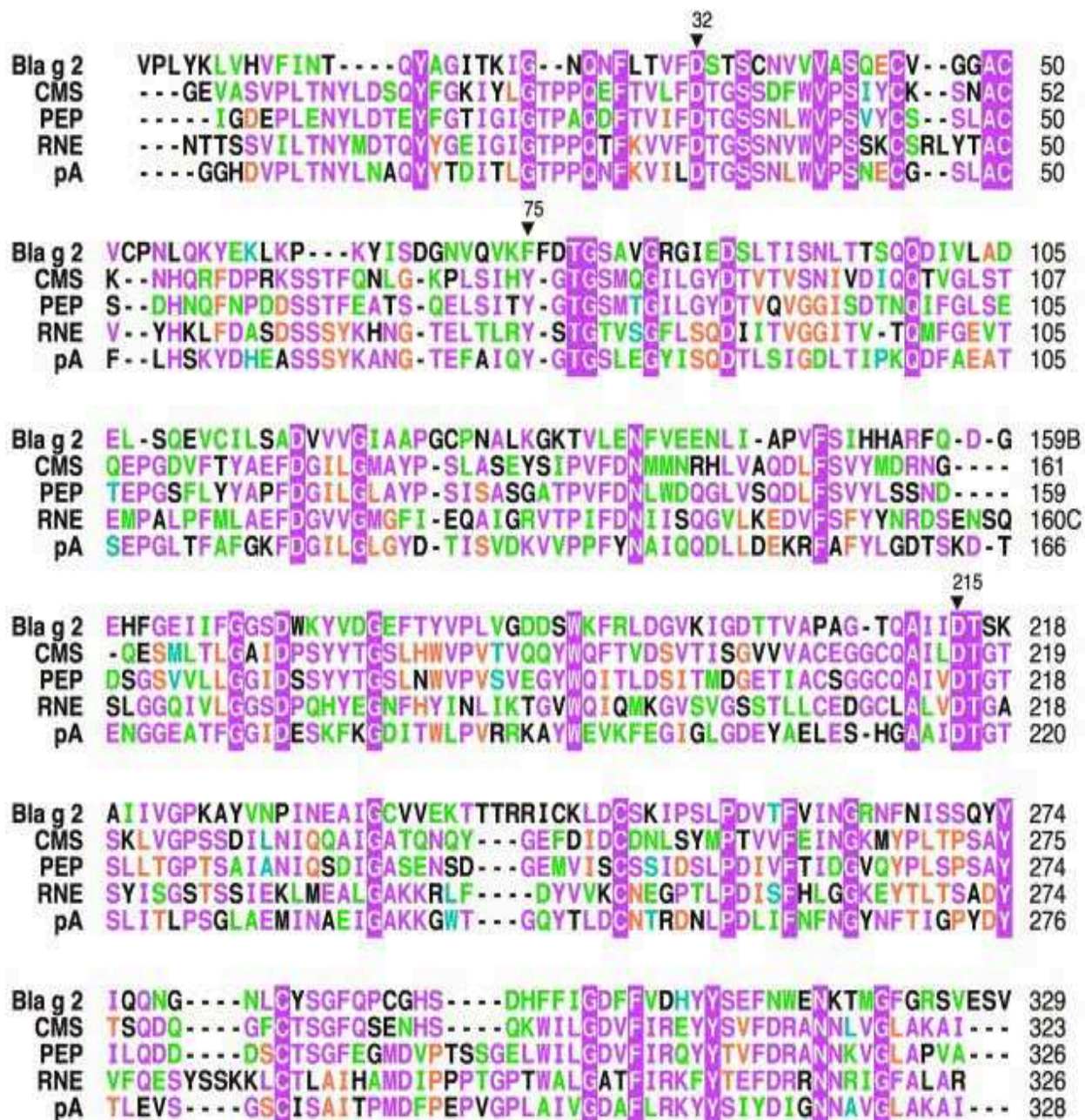


Figure 2. Structure-based sequence alignment comparing Bla g 2 with porcine pepsin (PEP), bovine chymosin (CMS), human renin (RNE), and yeast proteinase A (pA). White letters on the pink background indicate residues that are conserved in all five enzymes; pink and orange letters are used for residues identical in two or more proteins; green and blue letters denote similar residue types. A unique residue type is shown in black.¹⁰

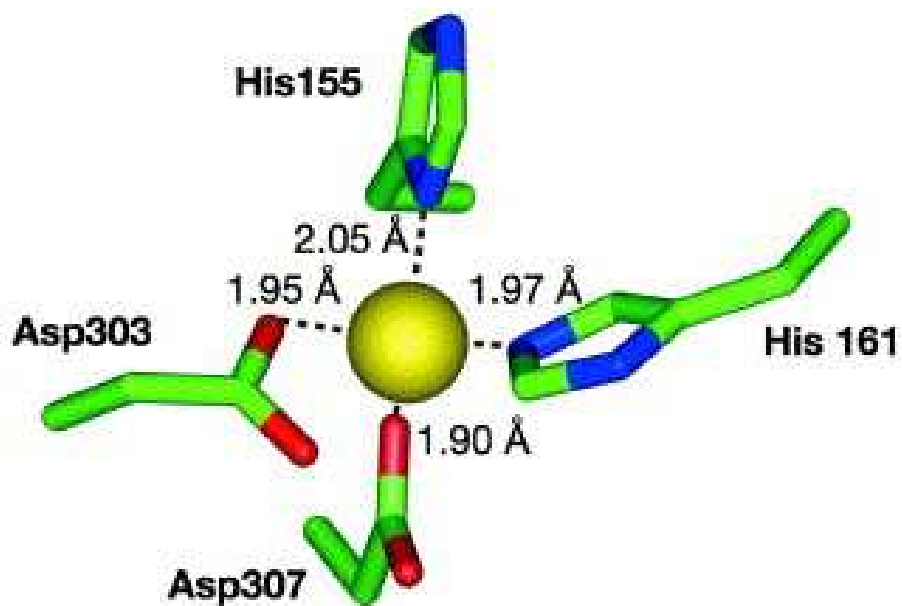


Figure 3. Metal-binding site in Bla g 2. A metal ion and the coordinating residues in Bla g 2. The zinc ion is shown as a ball, and protein residues in stick representation.¹⁰



Figure 4. Optimized SLIT film formulation

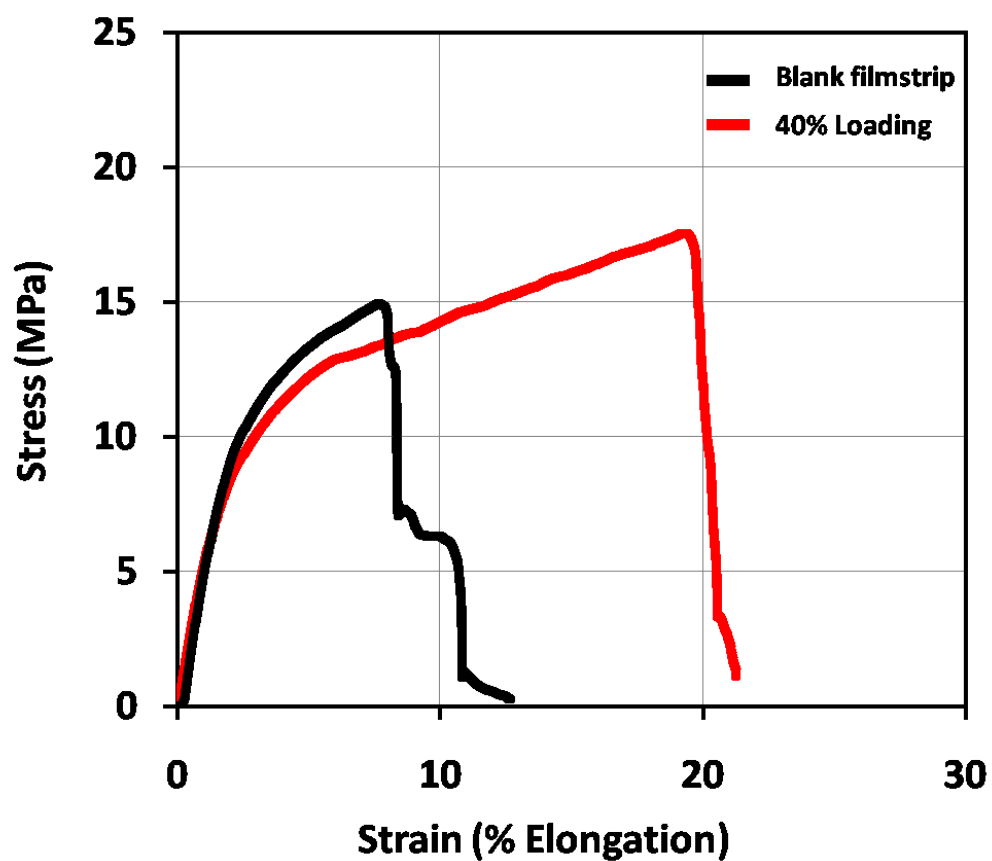


Figure 5. Typical Stress-Strain curve of a sublingual immunotherapy thin-filmstrip containing 40% aqueous cockroach protein extract (red). The black curve is that of a typical blank filmstrip without any allergen extract.

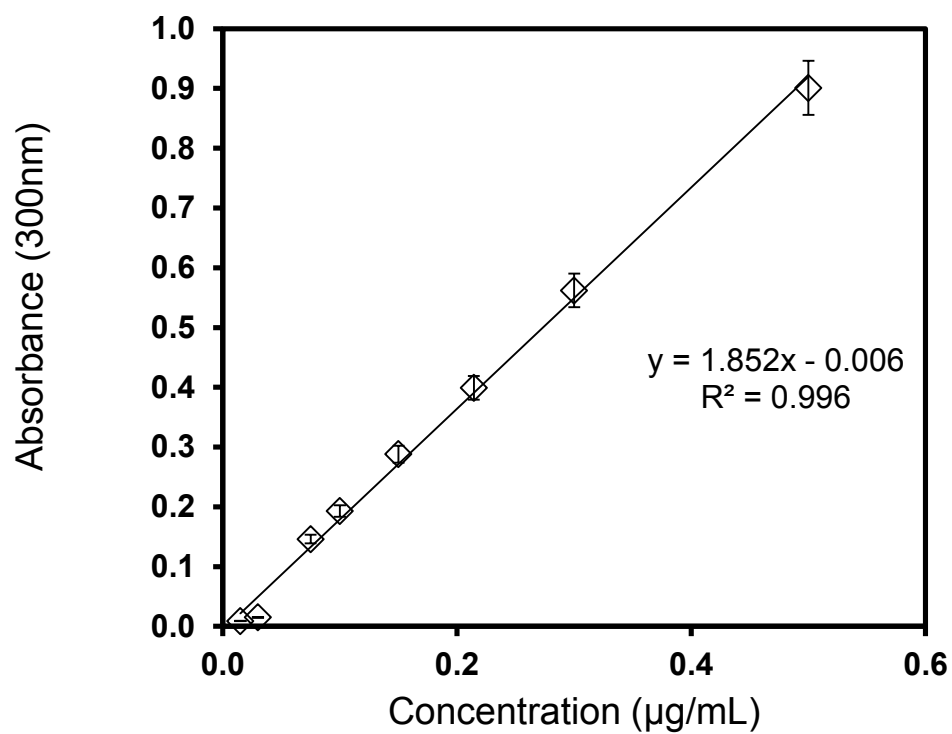


Figure 6. Serially diluted standard curve of cockroach aqueous protein extract. Standard concentrations range from 15 ng/mL to 1.5 µg/mL (n=3). UV/Visible spectroscopy was taken at 300nm. Slope of regression represents µg of total protein per film strip.

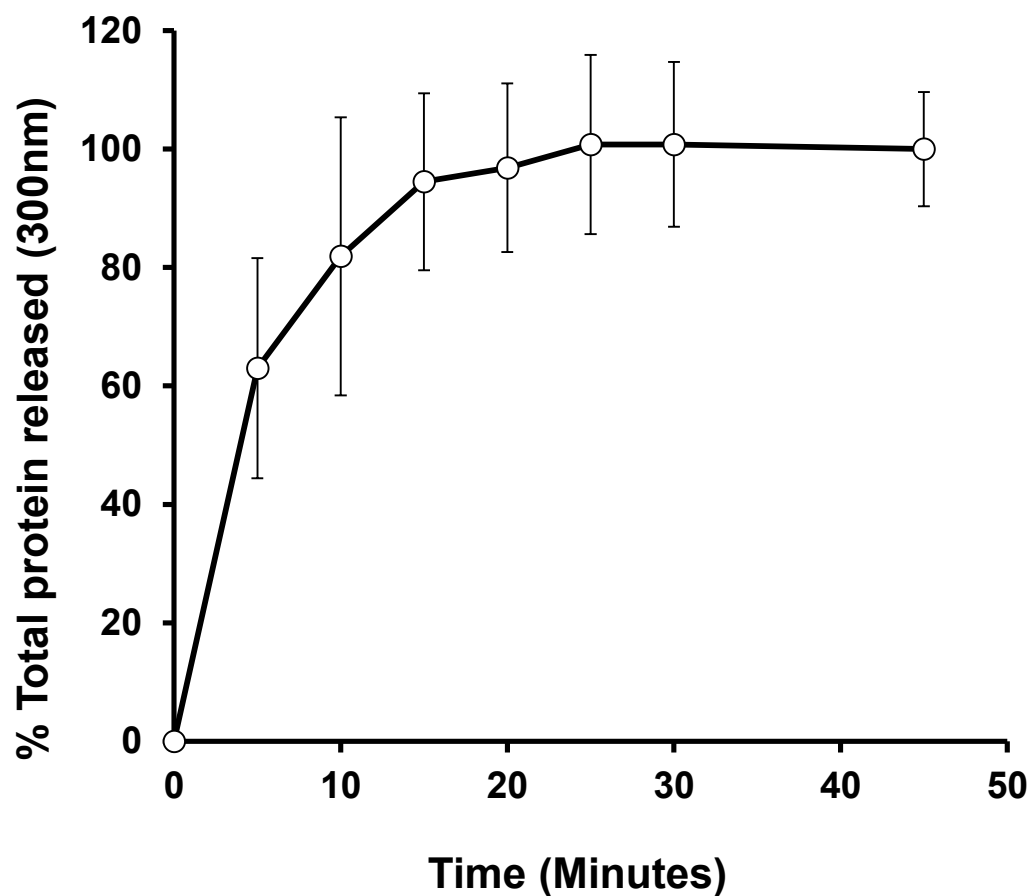


Figure 7. *In vitro* dissolution profile of sublingual immunotherapy filmstrips (n=6). Samplings times were 5, 10, 15, 20, 25, 30 and 45 minutes. The samples were analyzed on the UV/Vis spectroscopy at 300nm. The results were quantitated against the standard curve for the protein extract.

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OBJECTIVE

Pursue a challenging career in the field of pharmaceuticals and biotechnology, to fully utilize my trainings in the areas of analytical sciences as well as drug development and delivery.

PROFESSIONAL SUMMARY:

- Master of Science in Engineering with thesis training in both nanotechnology and gene delivery, as well as formulation development and characterization
- Results-oriented Analytical Scientist with parallel training in Quality Assurance, at a cGMP-compliant pharmaceutical Development & Manufacturing facility
- Strong analytical and problem solving skills with instrumental techniques
- Team player, with proven ability to work under pressure to meet deadlines

EDUCATION

Master of Science in Engineering (Thesis)

Johns Hopkins University

Materials Science and Engineering/Biomedical Engineering

May 2014

Thesis: The development and characterization of sublingual immunotherapy (SLIT) films for the treatment of cockroach allergies in inner city populations

Honors Bachelor of Science (Thesis)

University of Toronto

Pharmaceutical Chemistry

2005 - 2009

Thesis: Sensitivity of *Bacillus subtilis* endospores to a combination of acidic pH, antibacterial agent and pulsed high pressurization

SKILLS

Chromatography

HPLC, UPLC, GC, GPC (*Gel Permeation Chromatography*)

Spectroscopy

Mass Spectrometry (LC-MS), UV-VISIBLE, FTIR (*Infra-red*),
FID (*Flame Ionization*), DAD (*Diode Array*), RI (*Refractive Index*),
NMR (*Nuclear Magnetic Resonance*), Fluorescence Spectroscopy

Characterization

ELISA, BCA and Bradford protein assays, Dynamic Light Scattering (DLS), Surface Charge, Karl Fischer Moisture Analysis, Rheological Analysis, Disintegration and Dissolution

Microscopy

Transmission Electron Microscopy, Confocal Microscopy

PROFESSIONAL EXPERIENCE

Johns Hopkins University, Materials Science & Engineering

Baltimore, MD

Master of Science in Engineering

Jan 2013-May 2014

- Thesis Project
 - Developed Sublingual immunotherapy (SLIT) thin films for the treatment of cockroach allergies in children in urban households
 - Characterized the SLIT films for potency and stability using ELISA and LC-MS
 - Performed SLIT film testing following USP compendia methods
 - Optimized methods for the extraction of allergenic proteins from raw materials
- Additional Project
 - Designed morphology-controlled nanoparticles using DNA and siRNA, combined with a unique selection of polymers for gene therapies

- Expertise in DNA/siRNA nanoparticles characterization, such as particle size and surface charge, release kinetics, stability, Critical Micelle Concentration, protein adsorption and cell uptake
- Synthesized and characterized polymers used in nanoparticles formation

Capsugel Inc. –Formerly a division of Pfizer Inc.

Scientist, Analytical Development

Cambridge, MA

July 2011-July 2012

Assistant Scientist, Analytical Development

June 2010-July 2011

- Analytical Development and Validation
 - Executed studies including Assay, Related Substances, Content Uniformity, Dissolution, Specific Gravity and Water Content for drug substance, In-Process-Control samples and final products
 - Developed new methods or optimized existing methods in support of Batch Manufacturing campaigns and Regulatory studies
 - Led chemical and physical stability programs in support of Regulatory filings
 - Responsible for method transfer, verification and validation studies
 - Proficient in writing analytical methods and method validation protocols and associated reports
 - Developed and characterized lipid-based Self Micro-(and Nano) Emulsifying Drug Delivery Systems (SMEDDS / SNEDDS) for hard gelatin and HPMC capsules
 - Compiled phase diagrams for the development of the Lipidex® software
- Quality Assurance
 - Led Laboratory Investigations on Out-of-Specification (OOS) or Out-of-Trend (OOT) analytical results; compiled Investigation, Deviation & Change Control reports
 - Executed raw materials release testing following USP compendia methods
 - Generated release and sampling specifications following appropriate USP/EP guidelines
 - Assisted as auditor-in-training during internal audits

Posters:

- Venkatanarayanan J., Chen M., Gilicky O., Faraci J., Faraci W. *Detection and characterization of PEG excipients in drug formulations by FTIR.. Poster presented at Pittcon 2011; Atlanta, USA*
- Grieco E., Bacena J., Batheja P., Chen M., Gilicky O., Tran C., Venkatanarayanan J., Faraci W., Saxena V. *A Licaps® Drug Delivery System for Oral Administration of Paclitaxel. Poster presented at AAPS 2010; San Francisco, USA*

University of Toronto, Faculty of Pharmacy

Research Assistant

Toronto, ON

2007- 2009

- Designed and executed experiments for the sterilization of *Bacillus subtilis* using a high pressurization system
- Cultured microorganisms in controlled and sterilized environments
- Performed sterility testing, cell/endospore count and cell viability testing

University of Toronto, Department of Chemistry

Research Assistant

Toronto, ON

2005- 2008

- Studied and later independently performed the fabrication technique of microfluidic devices using the soft lithography technique
- Synthesized biopolymer microcapsules for cell encapsulation
- Skilled in instruments including the plasma cleaner and fluorescence microscopy